The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to evaluate the ability of fusion proteins of the invention to act on hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5.\beta_1$ and $\alpha_4.\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and are responsible for stimulating stem cell self-renewal havea not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of 0.2 μg/ cm^2. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Albumin fusion proteins of the invention are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where volume of the administed composition containing the albumin fusion protein of the invention represents 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2, 7% O_2, and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of albumin fusion proteins and polynucleotides of the invention (e.g., gene therapy).

If a particular fusion protein of the present invention is found to be a stimulator of hematopoietic progenitors, the fusion protein and polynucleotides corresponding to the fusion protein may be useful for example, in the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The fusion protein may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the albumin fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 39: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

An albumin fusion protein of the invention is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the fusion protein on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 µl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 µg/ml hEGF, 5mg/ml insulin, 1µg/ml hFGF, 50mg/ml gentamycin, 50 µg/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37 °C until day 2.

On day 2, serial dilutions and templates of an albumin fusion protein of the invention

are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or an albumin fusion protein of the invention and incubate at 37 degrees C/5% CO_2 until day 5.

Transfer 60μl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4 degrees C until Day 6 (for IL6 ELISA). To the remaining 100 μl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10μl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels.

Add 100 μl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the albumin fusion protein may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of the fusion protein and polynucleotides encoding the albumin fusion protein. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, fusion proteins may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, fusion proteins showing antagonistic activity in this

assay may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular agent (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as; for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, albumin fusion proteins that act as antagonists in this assay may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

Example 40: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing (containing an albumin fusion protein of the invention) and positive or negative controls are added to the plate in triplicate (in 10 μl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is

removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 μl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μl of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 41: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37 degreesC overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of an albumin fusion protein of the invention or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of

stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form (i.e., stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity). The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 42: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by fusion proteins of the invention. Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by the albumin fusion proteins that inhibit MLR since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Albumin fusion proteins of the invention found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2 x 10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2 x 10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate.

Dilutions of the fusion protein test material (50 µl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 µg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 µC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the fusion protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

Example 43: Assays for Protease Activity

The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacryamide/0.1% SDS gels containing 1% gelain orcasein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis apear as clear areas agains the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mM$NaPO_4$,1mM EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

Example 44: Identifying Serine Protease Substrate Specificity

Methods known in the art or described herein may be used to determine the substrate

specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

Example 45: Ligand Binding Assays

The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

Example 46: Functional Assay in Xenopus Oocytes

Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/mi. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocytc) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca2+ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 47: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus

capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

Example 48: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 49: ATP-binding assay

The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (^{32}P-ATP) (5 mCi/μmol, ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenly-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

Example 50: Phosphorylation Assay

In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein

incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P-ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion portein of the invention is incubated with the protein substrate, ^{32}P-ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P-ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

Example 51: Detection of Phosphorylation Activity (Activation) of an Albumin Fusion Protein of the Invention in the Presence of Polypeptide Ligands

Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

Example 52: Identification Of Signal Transduction Proteins That Interact With An albumin fusion protein Of The Present Invention

Albumin fusion proteins of the invention may serve as research tools for the identification, characterization and purification of signal transduction pathway proteins or receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 53: IL-6 Bioassay

A variety of assays are known in the art for testing the proliferative effects of an albumin fusion protein of the invention. For example, one such asssay is the IL-6 Bioassay as described by Marz *et al.* (*Proc. Natl. Acad. Sci., U.S.A., 95*:3251-56 (1998), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls

containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μl, and 50 μl of fusion protein of the invention is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention) relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

Example 54: Support of Chicken Embryo Neuron Survival

To test whether sympathetic neuronal cell viability is supported by an albumin fusion protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A., 96*:11458-63 (1998), which is herein incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO_2 in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mosmann, T., *J. Immunol. Methods, 65*:55-63 (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

Example 55: Assay for Phosphatase Activity

The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [^{32}P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-labeled MyBP.

Example 56: Interaction of Serine/Threonine Phosphatases with other Proteins

Fusion protein of the invention having serine/threonine phosphatase activity (e.g., as determined in Example 55) are useful, for example, as research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein -complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 57: Assaying for Heparanase Activity

There a numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells (1×10^6 cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S-labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

Example 58: Immobilization of biomolecules

This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constucts (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation

upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO4 and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl2, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

Example 59: Assays for Metalloproteinase Activity

Metalloproteinases are peptide hydrolases which use metal ions, such as Zn^{2+}, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

Proteolysis of alpha-2-macroglobulin

To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases

Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND $HgCl_2$), peptide metalloproteinase inhibitors (TIMP-1 and TIMP–2), and commercial small molecule MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC_{50} = 1.0 μM against MMP-1 and MMP–8; IC_{50} = 30 μM against MMP-9; IC_{50} = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC_{50} = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50μg/ml) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M

NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05%Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1 μl of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

Synthetic Fluorogenic Peptide Substrates Cleavage Assay

The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques knonw in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor-α (TNF-α) converting enzyme (TACE). These substrastes are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μM. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μl 1x HEPES buffer (0.2 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μl of substrate solution (50 μM) at 25 °C for 15 minutes, and then adding 20 μl of a purified fusion protein of the invention into the assay cuvett. The final concentration of substrate is 1 μM. Initial hydrolysis rates are monitored for 30-min.

Example 60:Identification and Cloning of VH and VL domains

One method to identfy and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent (Life Technologies, Rockville. MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75%

ethanol. Follwing washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is the dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can determined using optical density measurements.

cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transciptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 3. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerse, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes are stored 4°C.

Table 3: Primer Sequences Used to Amplify VH and VL domains.

Primer name	SEQ ID NO	Primer Sequence (5'-3')
VH Primers		
Hu VH1-5'	36	CAGGTGCAGCTGGTGCAGTCTGG
Hu VH2-5'	37	CAGGTCAACTTAAGGGAGTCTGG
Hu VH3-5'	38	GAGGTGCAGCTGGTGGAGTCTGG
Hu VH4-5'	39	CAGGTGCAGCTGCAGGAGTCGGG
Hu VH5-5'	40	GAGGTGCAGCTGTTGCAGTCTGC
Hu VH6-5'	41	CAGGTACAGCTGCAGCAGTCAGG
Hu JH1,2-5'	42	TGAGGAGACGGTGACCAGGGTGCC
Hu JH3-5'	43	TGAAGAGACGGTGACCATTGTCCC
Hu JH4,5-5'	44	TGAGGAGACGGTGACCAGGGTTCC
Hu JH6-5'	45	TGAGGAGACGGTGACCGTGGTCCC
VL Primers		
Hu Vkappa1-5'	46	GACATCCAGATGACCCAGTCTCC
Hu Vkappa2a-5'	47	GATGTTGTGATGACTCAGTCTCC
Hu Vkappa2b-5'	48	GATATTGTGATGACTCAGTCTCC
Hu Vkappa3-5'	49	GAAATTGTGTTGACGCAGTCTCC
Hu Vkappa4-5'	50	GACATCGTGATGACCCAGTCTCC
Hu Vkappa5-5'	51	GAAACGACACTCACGCAGTCTCC
Hu Vkappa6-5'	52	GAAATTGTGCTGACTCAGTCTCC
Hu Vlambda1-5'	53	CAGTCTGTGTTGACGCAGCCGCC
Hu Vlambda2-5'	54	CAGTCTGCCCTGACTCAGCCTGC
Hu Vlambda3-5'	55	TCCTATGTGCTGACTCAGCCACC
Hu Vlambda3b-5'	56	TCTTCTGAGCTGACTCAGGACCC
Hu Vlambda4-5'	57	CACGTTATACTGACTCAACCGCC
Hu Vlambda5-5'	58	CAGGCTGTGCTCACTCAGCCGTC
Hu Vlambda6-5'	59	AATTTTATGCTGACTCAGCCCCA
Hu Jkappa1-3'	60	ACGTTTGATTTCCACCTTGGTCCC
Hu Jkappa2-3'	61	ACGTTTGATCTCCAGCTTGGTCCC
Hu Jkappa3-3'	62	ACGTTTGATATCCACTTTGGTCCC
Hu Jkappa4-3'	63	ACGTTTGATCTCCACCTTGGTCCC
Hu Jkappa5-3'	64	ACGTTTAATCTCCAGTCGTGTCCC
Hu Jlambda1-3'	65	CAGTCTGTGTTGACGCAGCCGCC
Hu Jlambda2-3'	66	CAGTCTGCCCTGACTCAGCCTGC
Hu Jlambda3--3'	67	TCCTATGTGCTGACTCAGCCACC
Hu Jlambda3b-3'	68	TCTTCTGAGCTGACTCAGGACCC
Hu Jlambda4-3'	69	CACGTTATACTGACTCAACCGCC
Hu Jlambda5-3'	70	CAGGCTGTGCTCACTCAGCCGTC
Hu Jlambda6-3'	71	AATTTTATGCTGACTCAGCCCCA

PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc., Carlsbad, CA). Individual cloned PCR products can be isolated after transfection of E. coli and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors containing the nucleotide sequences of a heavy (e.g., human IgG1 or human IgG4) or light chain (human kappa or human lambda) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional antibody molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domain to generate expression vectors that encode complete antibody molecules are well known within the art.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through Identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety. The specification and sequence listing of each of the following U.S. applications are herein incorporated by reference in their entirety: Application Nos. 09/091,873 filed June 25, 1998; 60/229,358 filed on April 12, 2000; 60/199,384 filed on April 25,2000; 60/256,931 filed on December 21, 2000, 09/809,269, filed March 16, 2001; 60/277,980, filed March 23, 2001; 09/236,557, filed January 26, 1999; 09/482,273, filed January 13, 2000; 60/234,925, filed November 1, 2000; 09/397,945, filed September 17, 1999; 09/296,622, filed April 23, 1999; 60/092,921, filed July 15, 1998; 09/305,736, filed May 5, 1999; 09/781,417, filed February 13, 2001; 60/152,317, filed September 3, 1999; 09/227,357, filed January 8, 1999; and 60/262,066,

filed January 18, 2001; and International Publication Nos. WO98/39446, filed September 11, 1998; WO 00/61625, filed October 19, 2000; WO/00/77022, filed December 21, 2000; and WO/00/76530, filed December 21, 2000.

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NORWAY

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AUSTRALIA

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FINLAND

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The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: Unassigned

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What is claimed:

1. An albumin fusion protein comprising a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:18.

2. An albumin fusion protein comprising a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity.

3. The albumin fusion protein of claim 2, wherein said albumin activity is the ability to prolong the shelf life of the Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused state.

4. The albumin fusion protein of claim 2, wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:18.

5. An albumin fusion protein comprising a fragment or variant of a Therapeutic protein:X, and albumin comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has a biological activity of the Therapeutic protein:X.

6. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin.

7. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin.

8. The albumin fusion protein of any one of claims 1-5, wherein the

Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin.

9. The albumin fusion protein of any one of claims 1-5, which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof.

10. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker.

11. The albumin fusion protein of any one of claims 1-8, wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant of albumin.

12. The albumin fusion protein of any one of claims 1-11, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the Therapeutic protein:X in an unfused state.

13. The albumin fusion protein of any one of claims 1-11, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

14. The albumin fusion protein of any one of claims 1-11, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

15. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

16. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising an amino acid sequence selected from the group consisting of:

(a) amino acids 54 to 61 of SEQ ID NO:18;

(b) amino acids 76 to 89 of SEQ ID NO:18;

(c) amino acids 92 to100 of SEQ ID NO:18;

(d) amino acids 170 to 176 of SEQ ID NO:18;

(e) amino acids 247 to 252 of SEQ ID NO:18;

(f) amino acids 266 to 277 of SEQ ID NO:18;

(g) amino acids 280 to 288 of SEQ ID NO:18;

(h) amino acids 362 to 368 of SEQ ID NO:18;

(i) amino acids 439 to 447 of SEQ ID NO:18;

(j) amino acids 462 to 475 of SEQ ID NO:18;

(k) amino acids 478 to 486 of SEQ ID NO:18; and

(l) amino acids 560 to 566 of SEQ ID NO:18.

17. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life of the Therapeutic

protein:X , or a fragment or variant thereof, in an unfused state.

18. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

19. The albumin fusion protein of claims 15 or 16 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

20. The albumin fusion protein of any one of claims 1-19, which is non-glycosylated.

21. The albumin fusion protein of any one of claims 1-19, which is expressed in yeast.

22. The albumin fusion protein of claim 21, wherein the yeast is glycosylation deficient.

23. The albumin fusion protein of claim 21 wherein the yeast is glycosylation and protease deficient.

24. The albumin fusion protein of any one of claims 1-19, which is expressed by a mammalian cell.

25. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

26. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein further comprises a secretion leader sequence.

27. A composition comprising the albumin fusion protein of any one of claims 1-26 and a pharmaceutically acceptable carrier.

28. A kit comprising the composition of claim 27.

29. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-26.

30. The method of claim 29, wherein the disease or disorder comprises indication:Y.

31. A method of treating a patient with a disease or disorder that is modulated by Therapeutic protein:X, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 1-26.

32. The method of claim 31, wherein the disease or disorder is indication:Y.

33. A method of extending the shelf life of Therapeutic protein:X comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin or a fragment or variant thereof, sufficient to extend the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

34. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-26.

35. A vector comprising the nucleic acid molecule of claim 34.

5

36. A host cell comprising the nucleic acid molecule of claim 35.

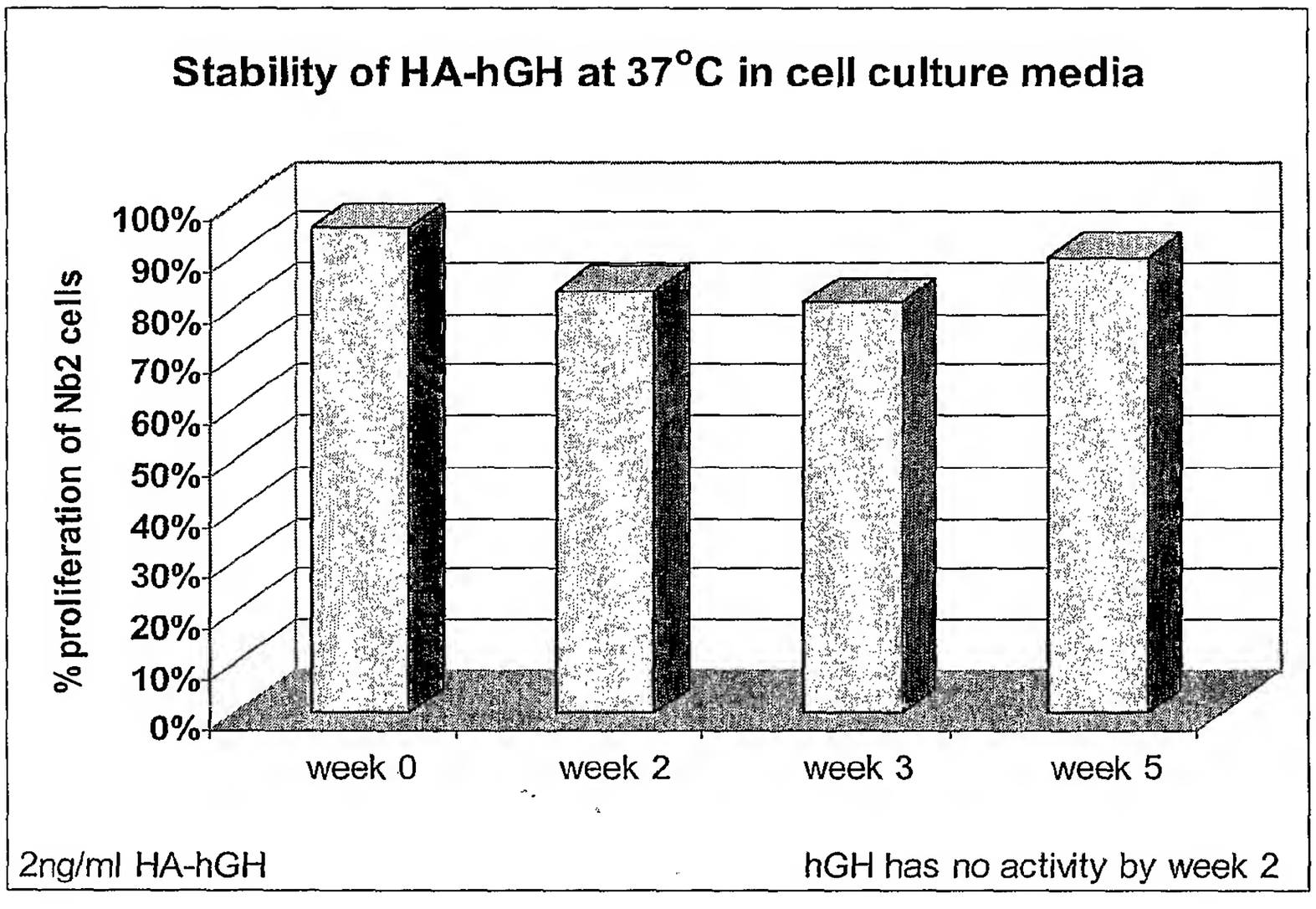

Stability of HA-hGH at 37°C in cell culture media
% proliferation of Nb2 cells
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
week 0
week 2
week 3
week 5
2ng/ml HA-hGH
hGH has no activity by week 2

Figure 1

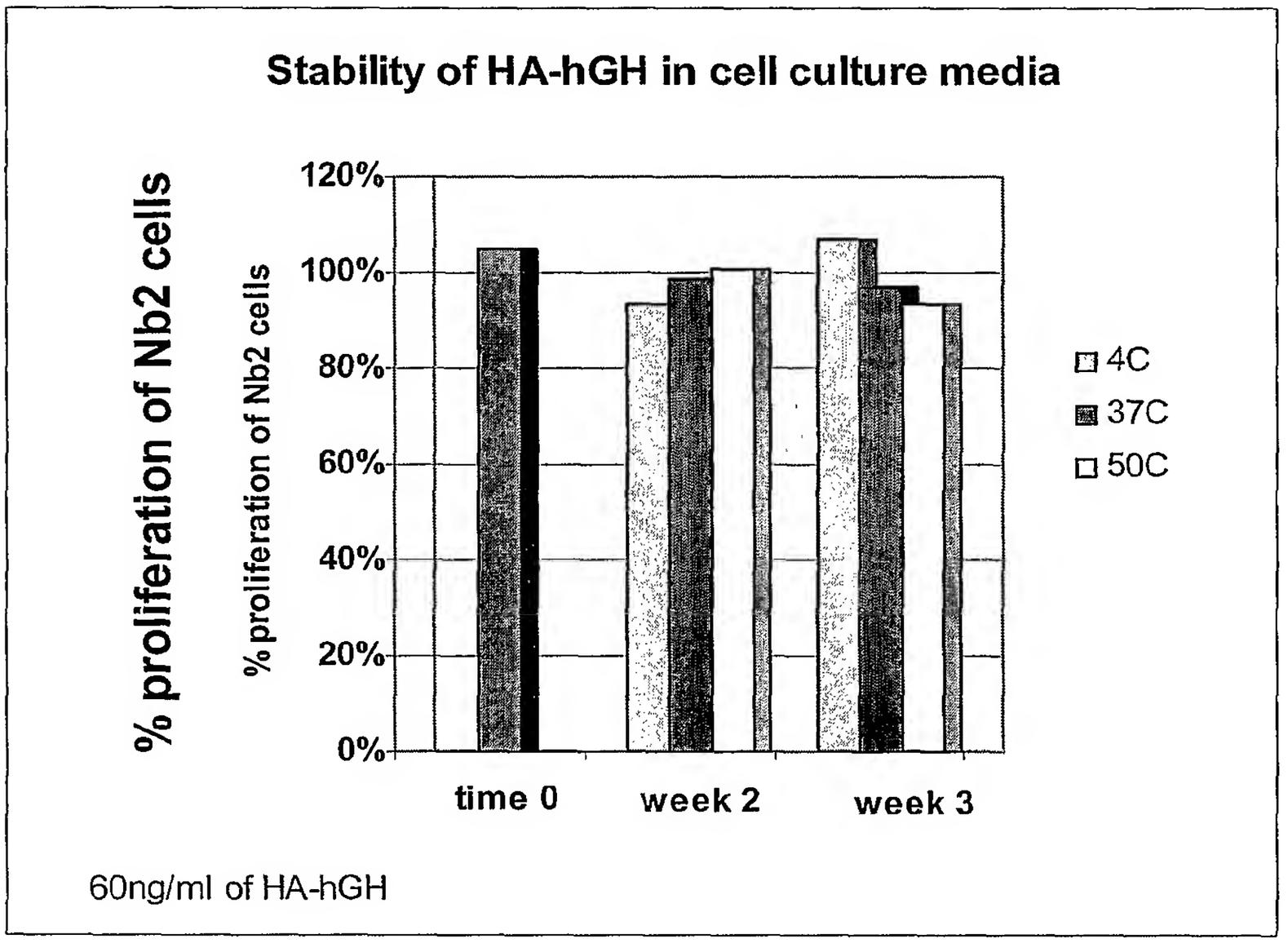

Stability of HA-hGH in cell culture media
% proliferation of Nb2 cells
% proliferation of Nb2 cells
120%
100%
80%
60%
40%
20%
0%
time 0
week 2
week 3
4C
37C
50C
60ng/ml of HA-hGH

Figure 2

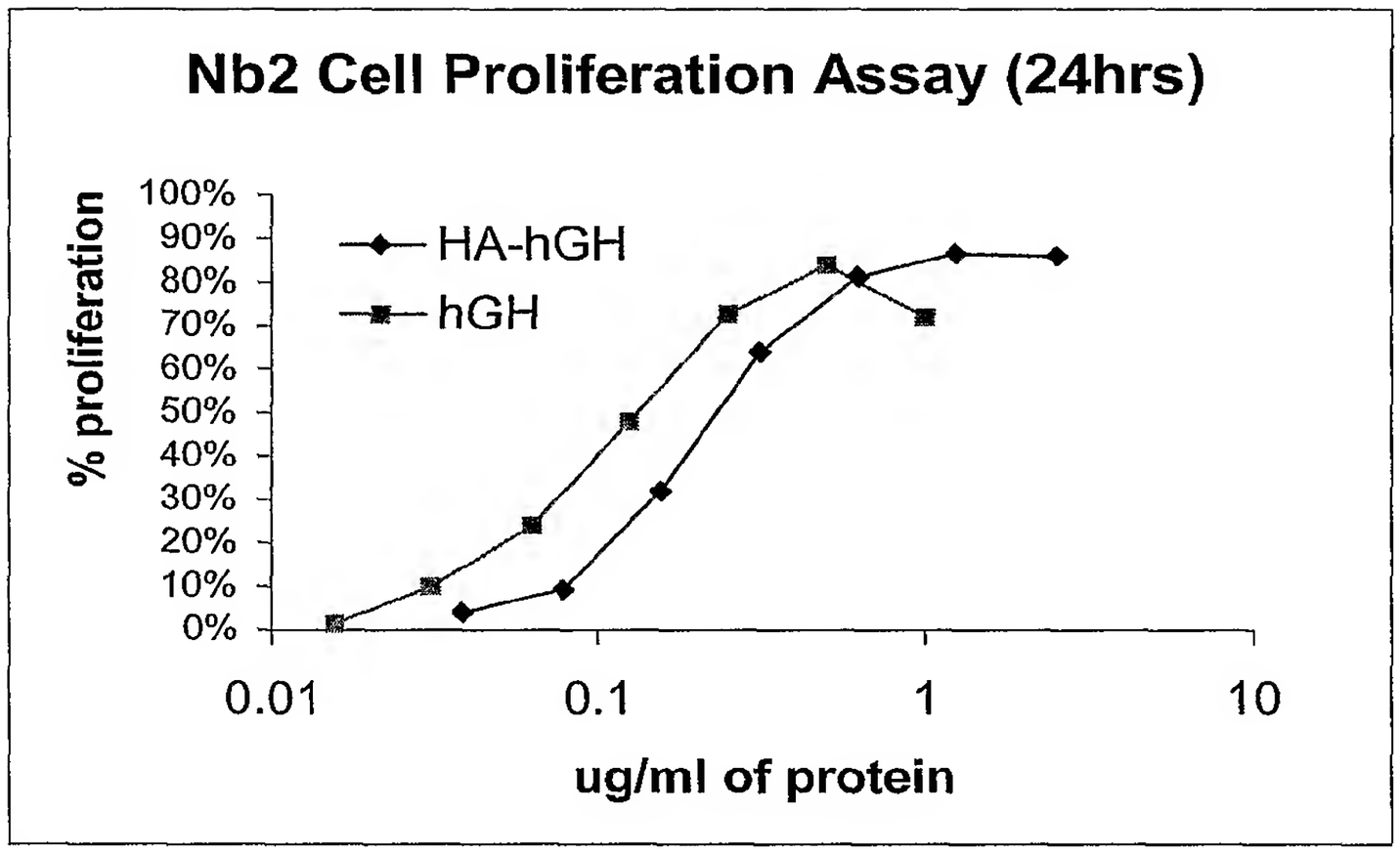


Figure 3A

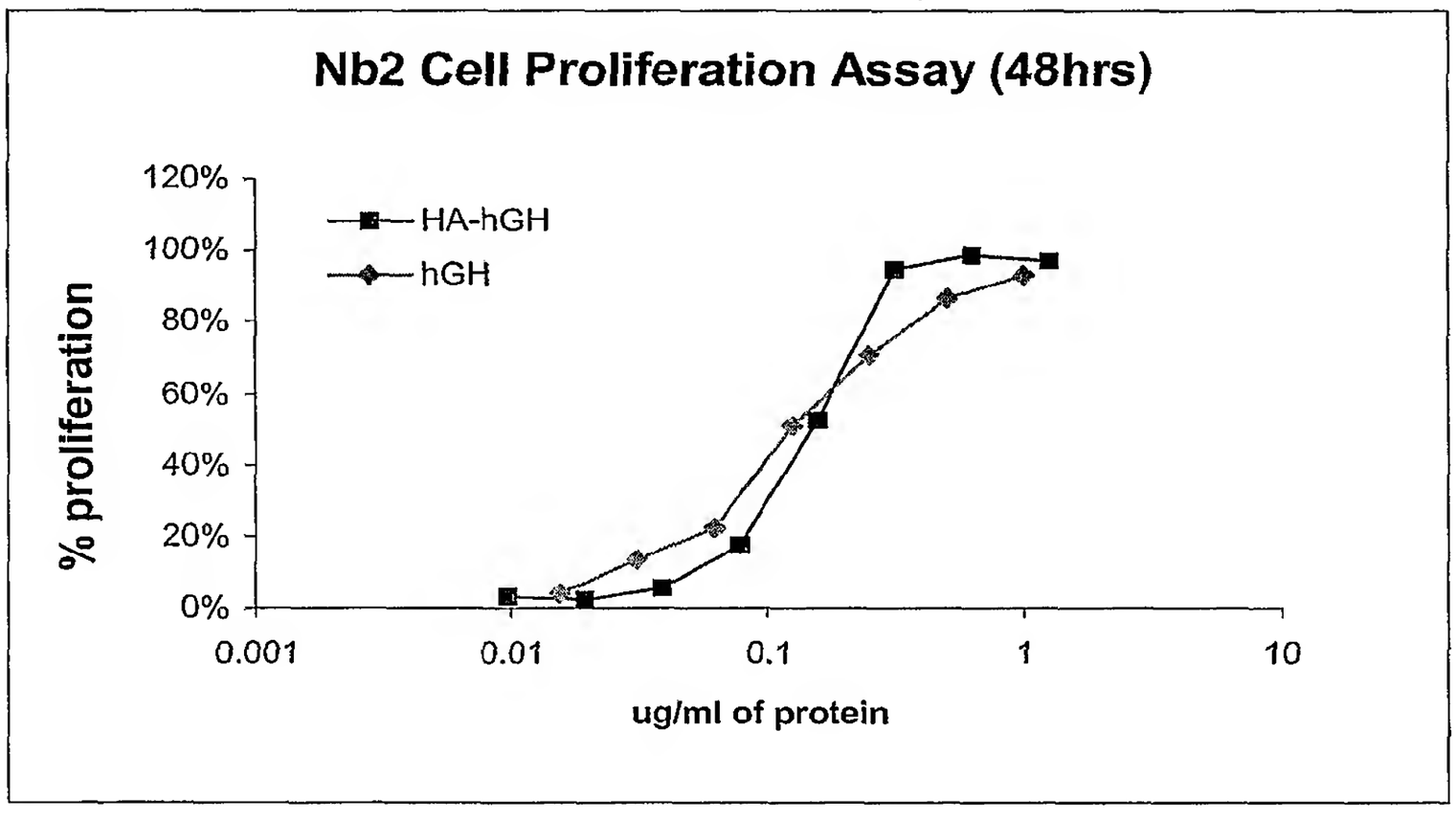


Figure 3B

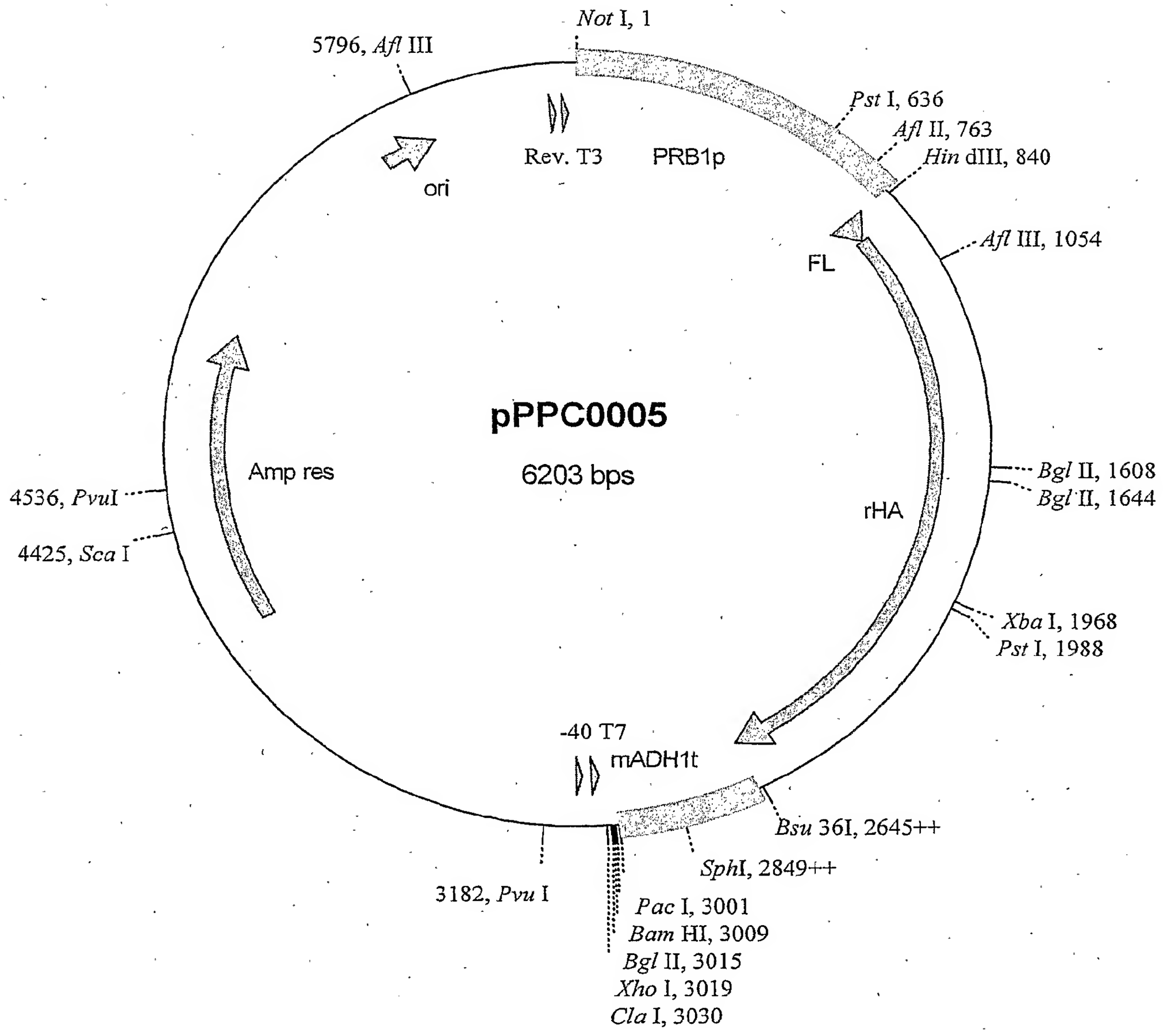

Not I, 1
5796, Afl III
Pst I, 636
Afl II, 763
Hin dIII, 840
Rev. T3
PRB1p
ori
Afl III, 1054
FL
pPPC0005
6203 bps
Amp res
Bgl II, 1608
Bgl II, 1644
rHA
4536, PvuI
4425, Sca I
Xba I, 1968
Pst I, 1988
-40 T7
mADH1t
Bsu 36I, 2645++
SphI, 2849++
3182, Pvu I
Pac I, 3001
Bam HI, 3009
Bgl II, 3015
Xho I, 3019
Cla I, 3030

Figure 4

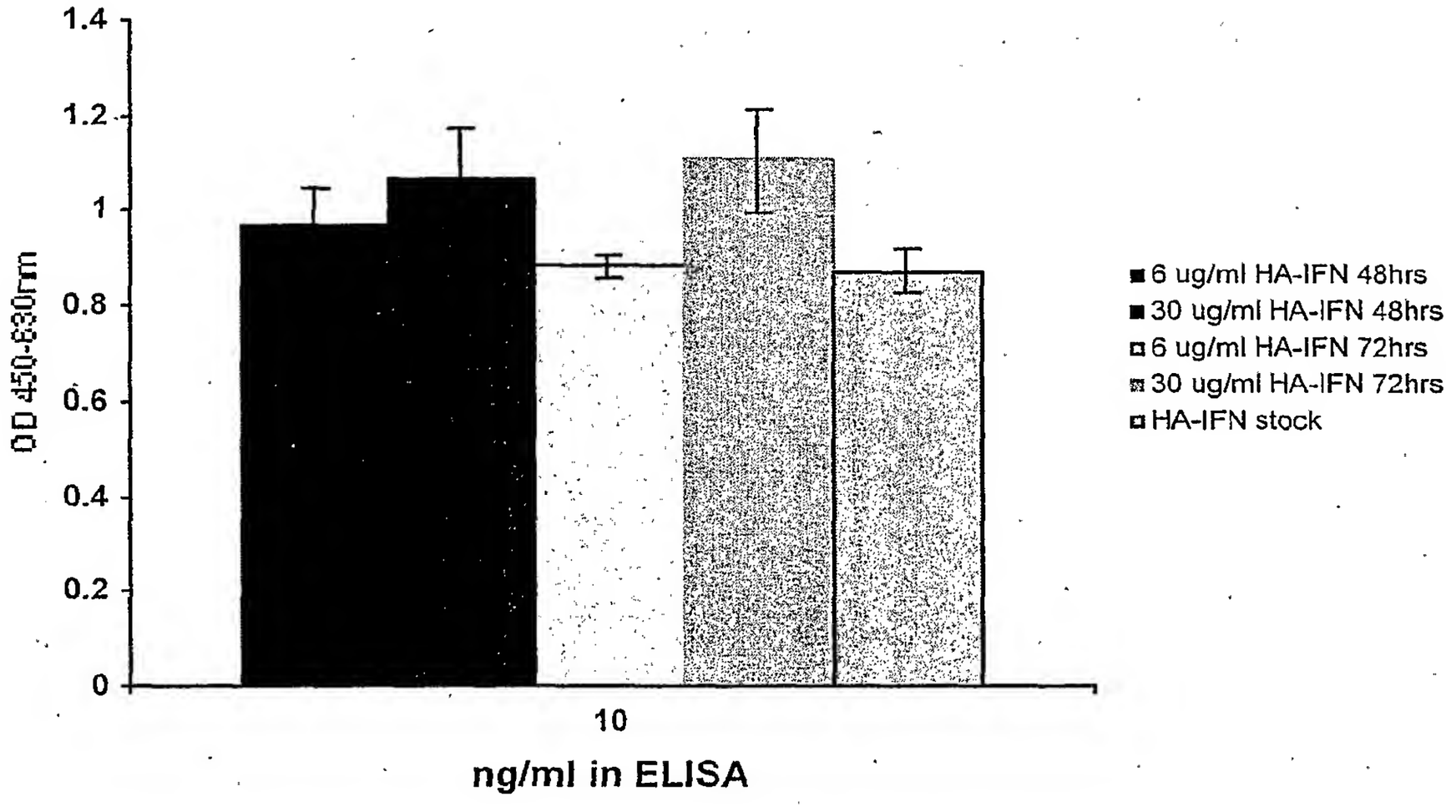

1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 450-630nm
10
ng/ml in ELISA
6 ug/ml HA-IFN 48hrs
30 ug/ml HA-IFN 48hrs
6 ug/ml HA-IFN 72hrs
30 ug/ml HA-IFN 72hrs
HA-IFN stock

Figure 5

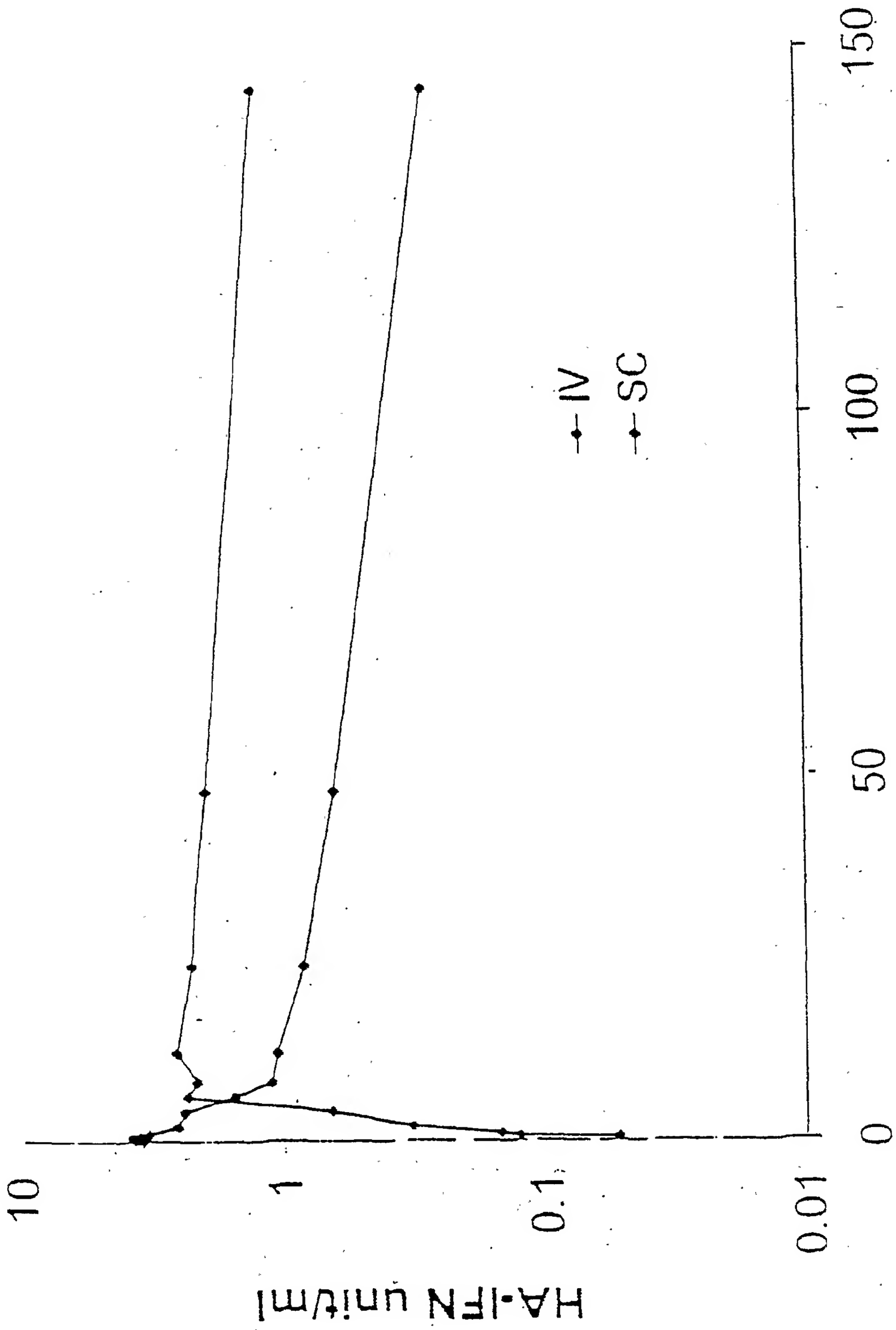

IV
SC
HA-IFN u/ml
10
1
0.1
0.01
0
50
100
150
Time after injection (hours)

Figure 6

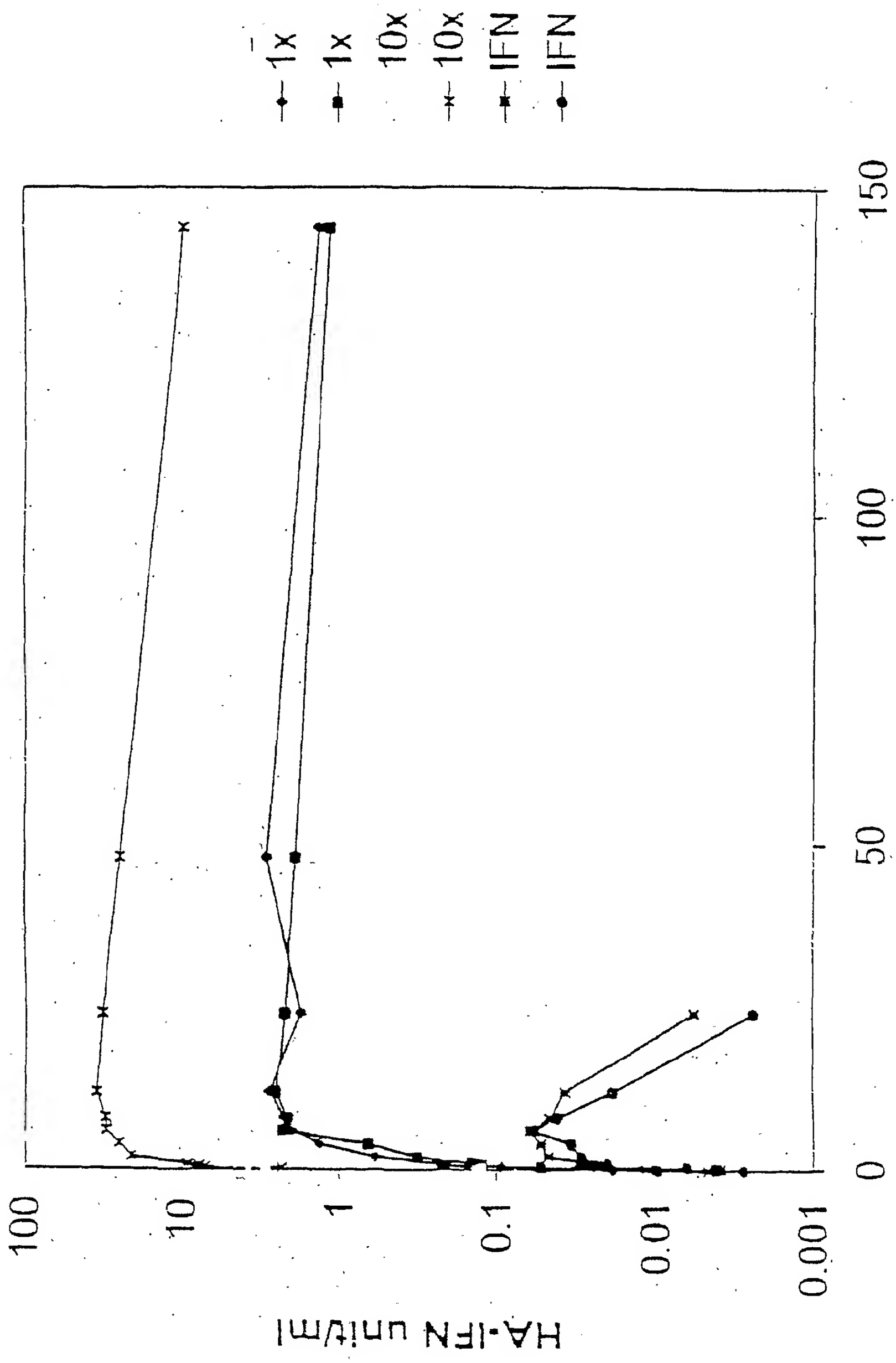

1x
1x
10x
10x
IFN
IFN
150
100
50
0
Hours after injection
100
10
1
0.1
0.01
0.001
0
HA-IFN unit/ml

Figure 7

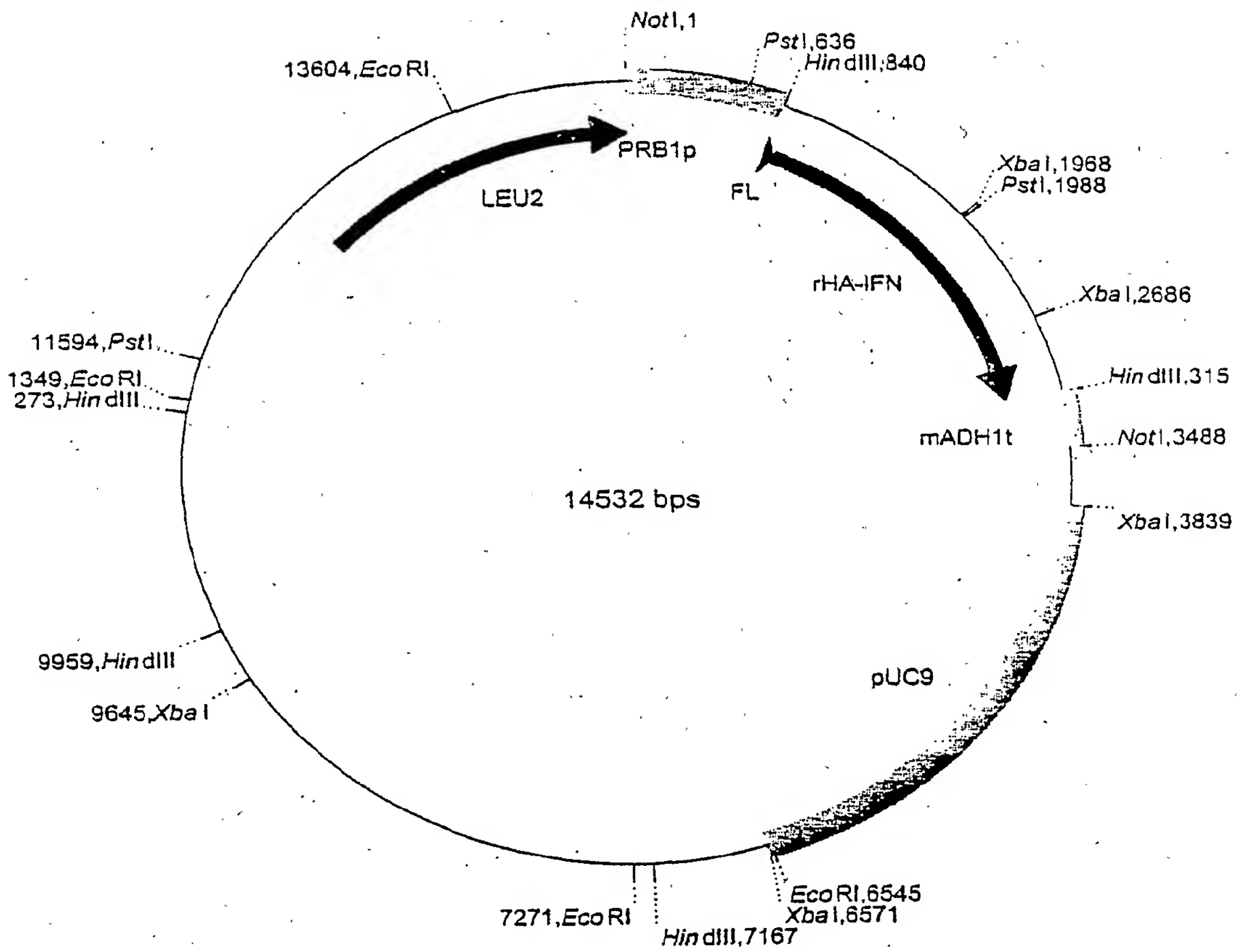


Figure 8. The HA-IFNα expression cassette in pSAC35. The expression cassette comprises
*PRB*1 promoter, from *S. cerevisiae.*
Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MFα-1 leader.
HA-IFNα coding sequence with a double stop codon (TAATAA)
*ADH*1 terminator, from *S. cerevisiae.* Modified to remove all the coding sequence normaly present in the *Hin*d III/*Bam*HI fragment generally used.

Figure 8

Localisation of 'Loops' based on the HA Crystal Structure which could be used for Mutation/Insertion

```
1    DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
          HHHHH HHH     HHH HHHHHHHHHH       HHHHH HHHHHHHHHH

                I                          II              III
51   KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKQEPERNE
     HHHHH              HHHHH HHHHH              HHHH H     HHHH

101  CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
     HHHH                 H HHHHHHHH   HHHHHHHHH HHHHH

                                IV
151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
     HHHHHHHHHH HHHHHHHHH      HHHHH HHHEHHHHHH HHHHHHHHHH

                                                          V
201  ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
     HHHHH   HH HHHHHHHHHH HH     HHH HHHHHHHHHH HHHHHH  HH

                           VI                VII
251  LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
     HHHHHHHHHH HHHHH          HHHHH    HHHHHHH H

301  DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
          HHHH     HHHHHH    HHHHHHH HHHHHH       HHHHHHHH

                     VIII
351  KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
     HHHHHHHHHH       HH    H   HHHHH HHHHHHHHHH HHHHHHH

                                                   IX
401  YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
     HHHHHHHHHH HHHH      H HHHHHHHHH    HHH        HHHHHHH

                          X                 XI
451  DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
     HHHHHHHHHH HHHHH      HHHHHHHHH      HHHHHHHH

501  EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
                   HHH   HHH HHHHMMEHHH HHH         HHHHHHH

                     XII
551  FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
     HHHHHHHH       HHHH HHHHHHHHHH HH
```

Loop		Loop	
I	Val54-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

```
                                IV
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHHHHHHHH HHHHHHHHHH

                                IV
151   APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHHHHHHHH HHHHHHHHHH
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

```
                    (X)n
                     ↓
                    IV

151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHHHHHHHH HHHHHHHHHH
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

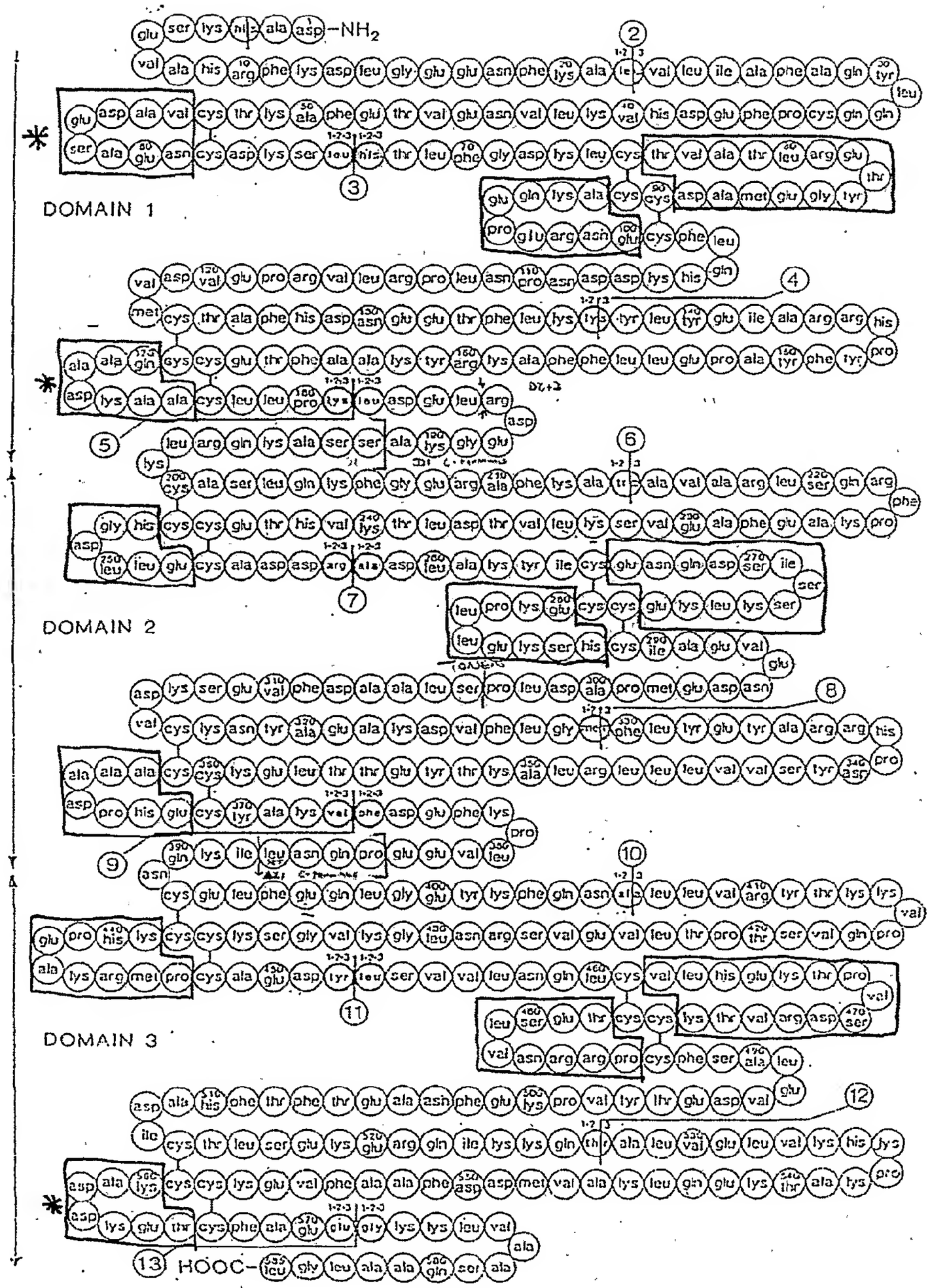
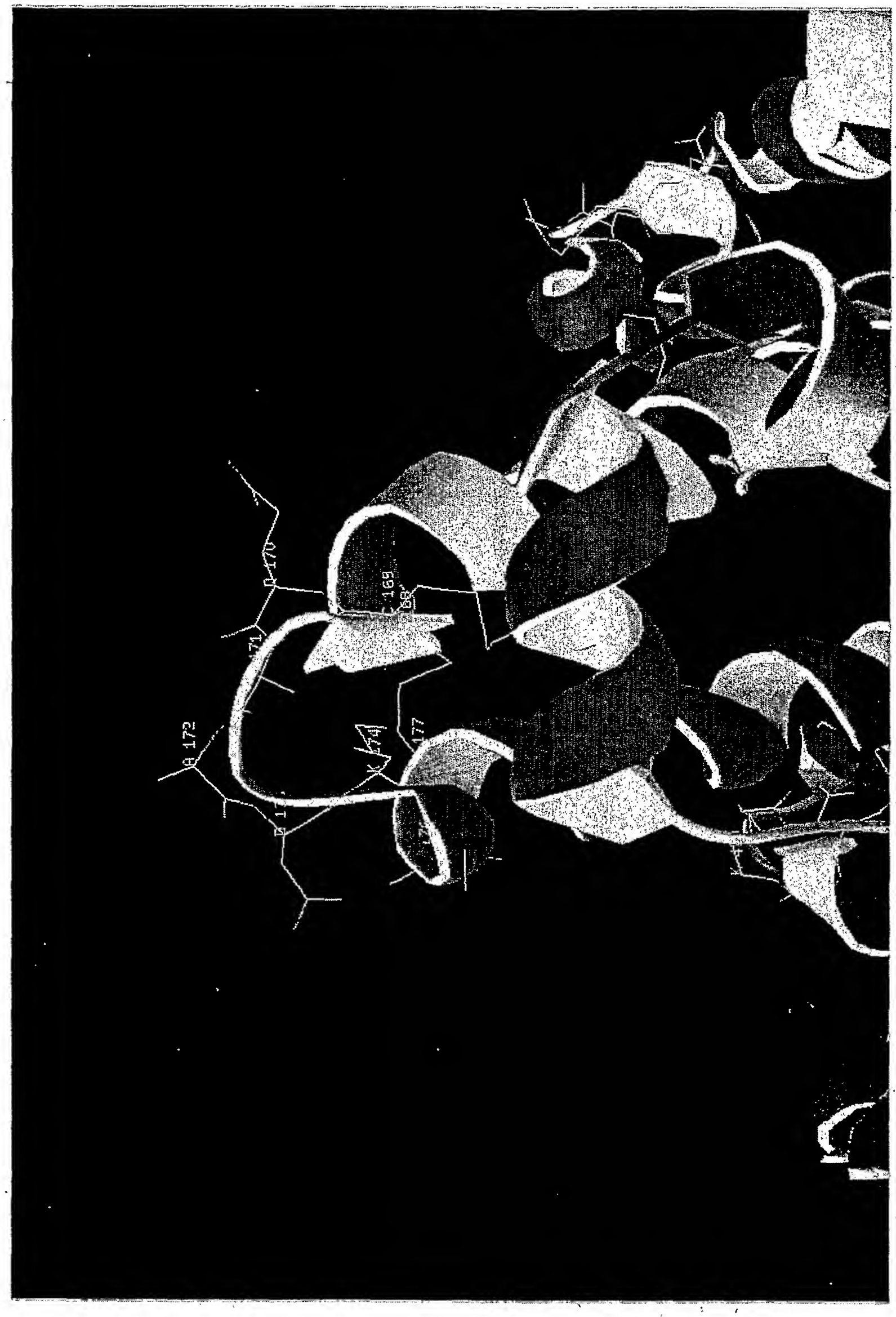


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

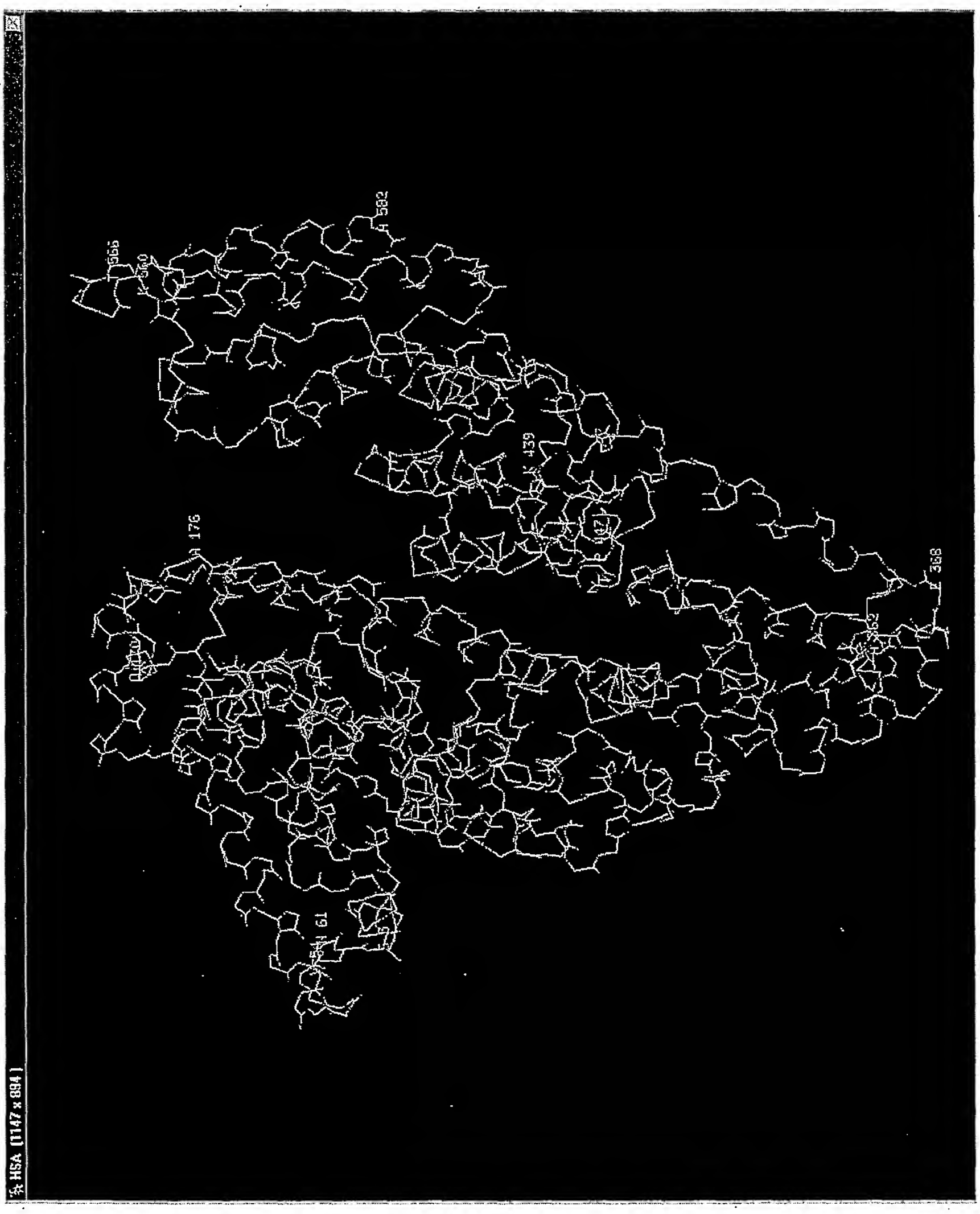


Figure 13: Tertiary Structure of HA

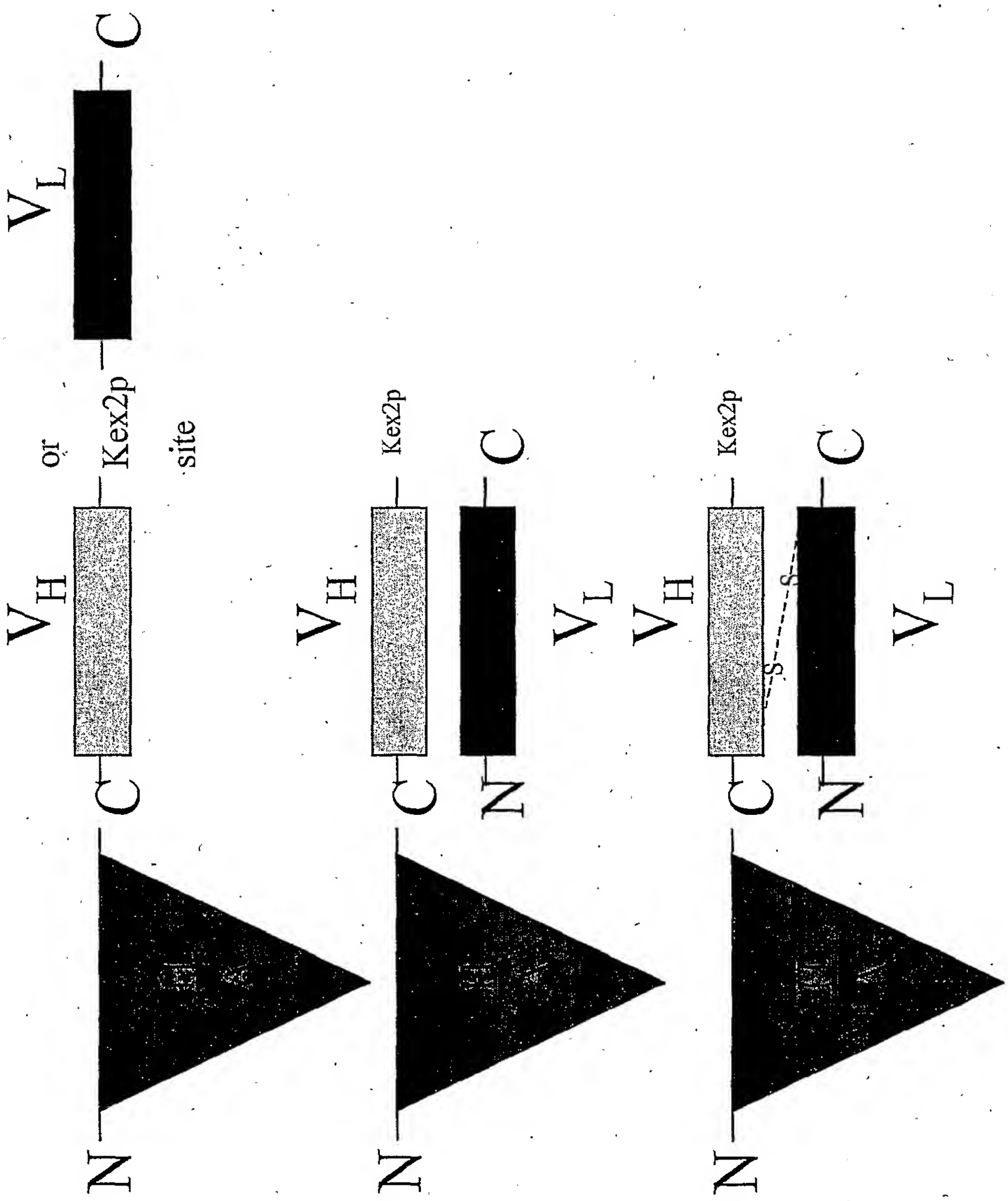


Figure 14: Schematic Diagram of Possible ScFv Fusions (Example is of a C-terminal fusion to HA)

```
  1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
  1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K   20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V   40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E   60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L   80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E   100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V   120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y   140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R   160
```

Figure 15A

```
481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y   K   A   A   F   T   E   C   C   Q   A   A   D   K   A   A   C   L   L   P   180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K   L   D   E   L   R   D   E   G   K   A   S   S   A   K   Q   R   L   K   C   200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A   S   L   Q   K   F   G   E   R   A   F   K   A   W   A   V   A   R   L   S   220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q   R   F   P   K   A   E   F   A   E   V   S   K   L   V   T   D   L   T   K   240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V   H   T   E   C   C   H   G   D   L   L   E   C   A   D   D   R   A   D   L   260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A   K   Y   I   C   E   N   Q   D   S   I   S   S   K   L   K   E   C   C   E   280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K   P   L   L   E   K   S   H   C   I   A   E   V   E   N   D   E   M   P   A   300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D   L   P   S   L   A   A   D   F   V   E   S   K   D   V   C   K   N   Y   A   320
```

Figure 15B

```
 961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E   A   K   D   V   F   L   G   M   F   L   Y   E   Y   A   R   R   H   P   D   340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y   S   V   V   L   L   L   R   L   A   K   T   Y   E   T   T   L   E   K   C   360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C   A   A   A   D   P   H   E   C   Y   A   K   V   F   D   E   F   K   P   L   380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V   E   E   P   Q   N   L   I   K   Q   N   C   E   L   F   E   Q   L   G   E   400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y   K   F   Q   N   A   L   L   V   R   Y   T   K   K   V   P   Q   V   S   T   420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P   T   L   V   E   V   S   R   N   L   G   K   V   G   S   K   C   C   K   H   440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P   E   A   K   R   M   P   C   A   E   D   Y   L   S   V   V   L   N   Q   L   460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C   V   L   H   E   K   T   P   V   S   D   R   V   T   K   C   C   T   E   S   480
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Figure 15C

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1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E   F   N   A   E   T   F   T   F   H   A   D   I   C   T   L   S   E   K   E   520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R   Q   I   K   K   Q   T   A   L   V   E   L   V   K   H   K   P   K   A   T   540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K   E   Q   L   K   A   V   M   D   D   F   A   A   F   V   E   K   C   C   K   560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A   D   D   K   E   T   C   F   A   E   E   G   K   K   L   V   A   A   S   Q   580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A   A   L   G   L   *                                   585
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Figure 15D

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Glu	Asn	Phe	Lys	Ala	Leu	Val	Leu	Ile	Ala	Phe	Ala	Gln	Tyr	Leu	Gln		
			20					25					30				
cag	tgt	cca	ttt	gaa	gat	cat	gta	aaa	tta	gtg	aat	gaa	gta	act	gaa		144
Gln	Cys	Pro	Phe	Glu	Asp	His	Val	Lys	Leu	Val	Asn	Glu	Val	Thr	Glu		
		35					40					45					
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Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala	Glu	Asn	Cys	Asp	Lys		
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tca	ctt	cat	acc	ctt	ttt	gga	gac	aaa	tta	tgc	aca	gtt	gca	act	ctt		240
Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Thr	Val	Ala	Thr	Leu		
65					70					75					80		
cgt	gaa	acc	tat	ggt	gaa	atg	gct	gac	tgc	tgt	gca	aaa	caa	gaa	cct		288
Arg	Glu	Thr	Tyr	Gly	Glu	Met	Ala	Asp	Cys	Cys	Ala	Lys	Gln	Glu	Pro		
				85					90					95			
gag	aga	aat	gaa	tgc	ttc	ttg	caa	cac	aaa	gat	gac	aac	cca	aac	ctc		336
Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Asn	Leu		
			100					105					110				
ccc	cga	ttg	gtg	aga	cca	gag	gtt	gat	gtg	atg	tgc	act	gct	ttt	cat		384
Pro	Arg	Leu	Val	Arg	Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His		

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aga cat cct tac ttt tat gcc ccg gaa ctc ctt ttc ttt gct aaa agg   480
Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

tat aaa gct gct ttt aca gaa tgt tgc caa gct gct gat aaa gct gcc   528
Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

tgc ctg ttg cca aag ctc gat gaa ctt cgg gat gaa ggg aag gct tcg   576
Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
        180                 185                 190

tct gcc aaa cag aga ctc aaa tgt gcc agt ctc caa aaa ttt gga gaa   624
Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
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aga gct ttc aaa gca tgg gca gtg gct cgc ctg agc cag aga ttt ccc   672
Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
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aaa gct gag ttt gca gaa gtt tcc aag tta gtg aca gat ctt acc aaa   720
Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

gtc cac acg gaa tgc tgc cat gga gat ctg ctt gaa tgt gct gat gac   768
Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
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agg gcg gac ctt gcc aag tat atc tgt gaa aat cag gat tcg atc tcc   816
Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
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agt aaa ctg aag gaa tgc tgt gaa aaa cct ctg ttg gaa aaa tcc cac   864
Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca   912
Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
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tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct   960
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
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gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga   1008
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
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agg cat cct gat tac tct gtc gtg ctg ctg ctg aga ctt gcc aag aca   1056
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
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tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa   1104
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
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Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
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Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
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caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa   1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
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Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
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Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
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Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca   1488
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
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tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat   1536
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
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ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca   1584
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
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ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg   1632
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
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aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag   1680
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt   1728
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
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Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
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Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
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Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
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Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
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Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
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Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
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Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
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Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
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Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
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Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
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Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
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Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
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Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
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<400> 27
aggagcgtcg acaaaagann nnnnnnnnnn nnn 33

<210> 28
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for generation of albumin fusion protein in which the albumin moiety is c-terminal of the Therapeutic Protein

<220>
<221> misc feature
<222> (38)
<223> n equals a,t,g, or c

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<220>
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<222> (52)
<223> n equals a,t,g, or c

<400> 28
ctttaaatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nn 52

<210> 29
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> signal peptide of natural human serum albumin protein

<400> 29
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg
20

<210> 30
<211> 114
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> forward primer useful for generation of PC4:HSA albumin fusion VECTOR

<220>
<221> misc_feature
<222> (5)..(10)
<223> BamHI retsriction site

<220>
<221> misc_feature
<222> (11)..(16)
<223> Hind III retsriction site

<220>
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<222> (17)..(27)
<223> Kozak sequence

<220>
<221> misc_feature
<222> (25)..(97)
<223> cds natural signal sequence of human serum albumin

<220>
<221> misc_feature
<222> (75)..(81)
<223> XhoI restriction site

<220>
<221> misc_feature
<222> (98)..(114)
<223> cds first six amino acids of human serum albumin

<400> 30
tcagggatcc aagcttccgc caccatgaag tgggtaacct ttatttccct tctttttctc 60
tttagctcgg cttactcgag gggtgtgttt cgtcgagatg cacacaagag tgag 114

<210> 31
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for generation of
PC4:HSA albumin fusion VECTOR

<220>
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<222> (6)..(11)
<223> Asp718 restriction site

<220>
<221> misc_feature
<222> (12)..(17)
<223> EcoRI restriction site

<220>
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<222> (15)..(17)
<223> reverse complement of stop codon

<220>
<221> misc_feature
<222> (18)..(25)
<223> AscI restriction site

<220>
<221> misc_feature
<222> (18)..(43)
<223> reverse complement of DNA sequence encoding last 9 amino acids

<400> 31
gcagcggtac cgaattcggc gcgccttata agcctaaggc agc 43

<210> 32
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> forward primer useful for inserting Therapeutic protein into pC4:HSA vector

<220>
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<222> (29)
<223> n equals a,t,g, or c

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<222> (30)
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<222> (43)
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<220>
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<222> (44)
<223> n equals a,t,g, or c

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<222> (45)
<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (46)
<223> n equals a,t,g, or c

<400> 32
ccgccgctcg aggggtgtgt ttcgtcgann nnnnnnnnnn nnnnnn 46

<210> 33
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for inserting Therapeutic protein into pC4:HSA vector

<220>
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<222> (38)
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<222> (39)
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<400> 33
agtcccatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nnnnn 55

<210> 34
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Stanniocalcin signal peptide

<400> 34
Met Leu Gln Asn Ser Ala Val Leu Leu Leu Val Ile Ser Ala Ser
1 5 10 15

Ala

<210> 35
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Synthetic signal peptide

<400> 35
Met Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala Leu
1 5 10 15

Trp Ala Pro Ala Arg Gly
20

<210> 36
<211> 23
<212> DNA

<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate VH forward primer useful for
amplifying human VH domains

<400> 36
caggtgcagc tggtgcagtc tgg 23

<210> 37
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate VH forward primer useful for
amplifying human VH domains

<400> 37
caggtcaact taagggagtc tgg 23

<210> 38
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate VH forward primer useful for
amplifying human VH domains

<400> 38
gaggtgcagc tggtggagtc tgg 23

<210> 39
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate VH forward primer useful for
amplifying human VH domains

<400> 39
caggtgcagc tgcaggagtc ggg 23

<210> 40
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate VH forward primer useful for
amplifying human VH domains

<400> 40
gaggtgcagc tgttgcagtc tgc 23

<210> 41
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate VH forward primer useful for amplifying human VH domains

<400> 41
caggtacagc tgcagcagtc agg 23

<210> 42
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate JH reverse primer useful for amplifying human VH domains

<400> 42
tgaggagacg gtgaccaggg tgcc 24

<210> 43
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate JH reverse primer useful for amplifying human VH domains

<400> 43
tgaagagacg gtgaccattg tccc 24

<210> 44
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate JH reverse primer useful for amplifying human VH domains

<400> 44
tgaggagacg gtgaccaggg ttcc 24

<210> 45
<211> 24
<212> DNA

<213> Artificial Sequence

<220>
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amplifying human VH domains

<400> 45
tgaggagacg gtgaccgtgg tccc 24

<210> 46
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vkappa forward primer useful for
amplifying human VL domains

<400> 46
gacatccaga tgacccagtc tcc 23

<210> 47
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vkappa forward primer useful for
amplifying human VL domains

<400> 47
gatgttgtga tgactcagtc tcc 23

<210> 48
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vkappa forward primer useful for
amplifying human VL domains

<400> 48
gatattgtga tgactcagtc tcc 23

<210> 49
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
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amplifying human VL domains

<400> 49

gaaattgtgt tgacgcagtc tcc 23

<210> 50
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 50
gacatcgtga tgacccagtc tcc 23

<210> 51
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 51
gaaacgacac tcacgcagtc tcc 23

<210> 52
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
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<400> 52
gaaattgtgc tgactcagtc tcc 23

<210> 53
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 53
cagtctgtgt tgacgcagcc gcc 23

<210> 54
<211> 23
<212> DNA
<213> Artificial Sequence

<220>

<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 54
cagtctgccc tgactcagcc tgc 23

<210> 55
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 55
tcctatgtgc tgactcagcc acc 23

<210> 56
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 56
tcttctgagc tgactcagga ccc 23

<210> 57
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 57
cacgttatac tgactcaacc gcc 23

<210> 58
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 58
caggctgtgc tcactcagcc gtc 23

<210> 59

<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 59
aattttatgc tgactcagcc cca 23

<210> 60
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
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<223>Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 60
acgtttgatt tccaccttgg tccc 24

<210> 61
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
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<223>Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 61
acgtttgatc tccagcttgg tccc 24

<210> 62
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
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<223>Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 62
acgtttgata tccactttgg tccc 24

<210> 63
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 63
acgtttgatc tccaccttgg tccc 24

<210> 64
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 64
acgtttaatc tccagtcgtg tccc 24

<210> 65
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 65
cagtctgtgt tgacgcagcc gcc 23

<210> 66
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 66
cagtctgccc tgactcagcc tgc 23

<210> 67
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
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<223>Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 67
tcctatgtgc tgactcagcc acc 23

<210> 68
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Jlambda reverse primer useful for
amplifying human VL domains

<400> 68
tcttctgagc tgactcagga ccc 23

<210> 69
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
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amplifying human VL domains

<400> 69
cacgttatac tgactcaacc gcc 23

<210> 70
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
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amplifying human VL domains

<400> 70
caggctgtgc tcactcagcc gtc 23

<210> 71
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221>primer_bind
<223>Degenerate Jlambda reverse primer useful for
amplifying human VL domains

<400> 71
aattttatgc tgactcagcc cca 23

<210> 72
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221>turn
<223>Linker peptide that may be used to join VH
and VL domains in an scFv.

<400> 72
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

1 5 10 15

<210> 73
<211> 23
<212> PRT
<213> Homo sapiens

<400> 73
Cys Cys Cys Ala Ala Gly Ala Ala Thr Thr Cys Cys Cys Thr Thr Ala
1 5 10 15

Thr Cys Cys Ala Gly Gly Cys
20

<210> 74
<211> 429
<212> PRT
<213> Homo sapiens

<400> 74
Met Cys Pro Gly Ala Leu Trp Val Ala Leu Pro Leu Leu Ser Leu Leu
1 5 10 15

Ala Gly Ser Leu Gln Gly Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser
20 25 30

Ala Gly Gly Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu
35 40 45

Pro Glu His Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val
50 55 60

Asp Phe Leu Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys
65 70 75 80

Thr Arg Val Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr
85 90 95

Thr Ser Asp Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe
100 105 110

Ser Met Glu Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe
115 120 125

Gln Lys His Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln
130 135 140

Ile Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val
145 150 155 160

Asp Pro Ser His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu
165 170 175

Asp Gly Thr Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu
180 185 190

Val Ser Gln Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser
195 200 205

Ser Ala Val Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn

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    210                 215                 220

Lys Leu Glu Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu
225                 230                 235                 240

Asp Ile Ser Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val
                245                 250                 255

Phe Ser Asn Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu
            260                 265                 270

Arg Glu Met Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser
        275                 280                 285

Lys Asp Gly Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr
    290                 295                 300

Asp Gly His Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser
305                 310                 315                 320

Ala Gly Ala Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe
                325                 330                 335

Glu Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu
            340                 345                 350

Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val
        355                 360                 365

Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe
    370                 375                 380

Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro
385                 390                 395                 400

Ile Ser Val Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr
                405                 410                 415

His Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg
            420                 425
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<210> 75
<211> 280
<212> PRT
<213> Homo sapiens

<400> 75

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Met Ala Pro Ser Gly Ser Leu Ala Val Pro Leu Ala Val Leu Val Leu
  1                 5                  10                  15

Leu Leu Trp Gly Ala Pro Trp Thr His Gly Arg Arg Ser Asn Val Arg
            20                  25                  30

Val Ile Thr Asp Glu Asn Trp Arg Glu Leu Leu Glu Gly Asp Trp Met
        35                  40                  45

Ile Glu Phe Tyr Ala Pro Trp Cys Pro Ala Cys Gln Asn Leu Gln Pro
    50                  55                  60

Glu Trp Glu Ser Phe Ala Glu Trp Gly Glu Asp Leu Glu Val Asn Ile
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65 70 75 80

Ala Lys Val Asp Val Thr Glu Gln Pro Gly Leu Ser Gly Arg Phe Ile
85 90 95

Ile Thr Ala Leu Pro Thr Ile Tyr His Cys Lys Asp Gly Glu Phe Arg
100 105 110

Arg Tyr Gln Gly Pro Arg Thr Lys Lys Asp Phe Ile Asn Phe Ile Ser
115 120 125

Asp Lys Glu Trp Lys Ser Ile Glu Pro Val Ser Ser Trp Phe Gly Pro
130 135 140

Gly Ser Val Leu Met Ser Ser Met Ser Ala Leu Phe Gln Leu Ser Met
145 150 155 160

Trp Ile Arg Thr Cys His Asn Tyr Phe Ile Glu Asp Leu Gly Leu Pro
165 170 175

Val Trp Gly Ser Tyr Thr Val Phe Ala Leu Ala Thr Leu Phe Ser Gly
180 185 190

Leu Leu Leu Gly Leu Cys Met Ile Phe Val Ala Asp Cys Leu Cys Pro
195 200 205

Ser Lys Arg Arg Arg Pro Gln Pro Tyr Pro Tyr Pro Ser Lys Lys Leu
210 215 220

Leu Ser Glu Ser Ala Gln Pro Leu Lys Lys Val Glu Glu Glu Gln Glu
225 230 235 240

Ala Asp Glu Glu Asp Val Ser Glu Glu Glu Ala Glu Ser Lys Glu Gly
245 250 255

Thr Asn Lys Asp Phe Pro Gln Asn Ala Ile Arg Gln Arg Ser Leu Gly
260 265 270

Pro Ser Leu Ala Thr Asp Lys Ser
275 280

<210> 76
<211> 112
<212> PRT
<213> Homo sapiens

<400> 76
Met Phe Trp Val Met Glu Thr Ala Lys Pro Pro Val Ser Glu Asp Ser
1 5 10 15

Phe Arg Leu Pro Arg Lys Trp Gly Trp Arg Thr Glu Ala Thr Ala Pro
20 25 30

His Ala Pro Val Pro Gln Ser Ile Cys Pro Arg Tyr Thr Ser Pro Cys
35 40 45

Ala Pro His Asp Cys Gly Ser Gln Thr Val Gln Gly Asn Ser Leu Ser
50 55 60

Leu Phe Tyr Thr Leu Ser His Lys Ala Pro Gln Leu Pro His Arg Val

65 70 75 80

Pro Ala Pro Leu Phe Cys Lys Tyr Val Lys Arg Lys Lys Cys Lys Arg
85 90 95

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Met Lys Thr Val Lys Cys Ala Pro Gly Val Asp Val Cys Thr Glu Ala
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Val Gly Ala Val Glu Thr Ile His Gly Gln Phe Ser Leu Ala Val Arg
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Gly Cys Gly Ser Gly Leu Pro Gly Lys Asn Asp Arg Gly Leu Asp Leu
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His Gly Leu Leu Ala Phe Ile Gln Leu Gln Gln Cys Ala Gln Asp Arg
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Cys Asn Ala Lys Leu Asn Leu Thr Ser Arg Ala Leu Asp Pro Ala Gly
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Asn Glu Ser Ala Tyr Pro Pro Asn Gly Val Glu Cys Tyr Ser Cys Val
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Gly Leu Ser Arg Glu Ala Cys Gln Gly Thr Ser Pro Pro Val Val Ser
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165 170 175

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Phe Thr Leu Ser Gly Ser Cys Cys Gln Gly Ser Arg Cys Asn Ser Asp
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Leu Arg Asn Lys Thr Tyr Phe Ser Pro Arg Ile Pro Pro Leu Val Arg
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Leu Pro Pro Pro Glu Pro Thr Thr Val Ala Ser Thr Thr Ser Val Thr

245 250 255

Thr Ser Thr Ser Ala Pro Val Arg Pro Thr Ser Thr Thr Lys Pro Met
260 265 270

Pro Ala Pro Thr Ser Gln Thr Pro Arg Gln Gly Val Glu His Glu Ala
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Ser Arg Asp Glu Glu Pro Arg Leu Thr Gly Gly Ala Ala Gly His Gln
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Asp Arg Ser Asn Ser Gly Gln Tyr Pro Ala Lys Gly Gly Pro Gln Gln
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Pro His Asn Lys Gly Cys Val Ala Pro Thr Ala Gly Leu Ala Ala Leu
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<213> Homo sapiens

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Gln Val Cys Asn Trp Phe Ile Asn Ala Arg Arg Arg Leu Leu Pro Asp
85 90 95

Met Leu Arg Lys Asp Gly Lys Asp Pro Asn Gln Phe Thr Ile Ser Arg
100 105 110

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115 120 125

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Thr Ala Gly Pro Asn Pro Thr Leu Gly Arg Pro Leu Ser Pro Lys Pro
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Ser Ser Pro Gly Ser Val Leu Ala Arg Pro Ser Val Ile Cys His Thr
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Thr Val Thr Ala Leu Lys Asp Val Pro Phe Ser Leu Cys Gln Ser Val

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Gly Val Gly Gln Asn Thr Asp Ile Gln Gln Ile Ala Ala Lys Asn Phe
        195                 200                 205

Thr Asp Thr Ser Leu Met Tyr Pro Glu Asp Thr Cys Lys Ser Gly Pro
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Pro Asp Leu Asn Gln Asp Phe Ser Gly Phe Gln Leu Leu Val Asp Val
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<212> PRT
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<400> 79

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        35                  40                  45

Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu
    50                  55                  60

Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn
65                  70                  75                  80

Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln
                85                  90                  95

Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
            100                 105                 110

Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
        115                 120                 125

Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
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Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr
145                 150                 155                 160

Pro Thr Ser Cys Ser Arg Cys
                165
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11991

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 37/02; C12N 15/00
US CL : 530/350; 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
EST, GENEMBL, AGENESEQ

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE, BIOSIS, USPAT, JAPIO, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/23857 A1 (DELTA BIOTECHNOLOGY LIMITED) 08 September 1995 (08.09.95), see entire document.	1-9, 15-19
X	EP 0 322 094 A1 (DELTA BIOTECHNOLOGY LIMITED) 28 June 1989 (28.06.89), see entire document.	1-9, 15-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 JULY 2001	Date of mailing of the international search report 14 AUG 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HOPE ROBINSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11991

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-14, 20-32 and 34-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 15-19 (FGF-7)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11991

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Groups 1-58, claim(s) 1-9, 15-19, all in part, drawn to a therapeutic protein X, wherein X correlates to those listed in the Table on page 11 of the description. If any of Groups 1-58 are elected the claims will only be examined in-so-far-as it pertains to the elected protein X. For example,
If Group 1 is elected, this correlates to Therapeutic Protein X:FGF-7.
Group 2, all partially as in Group 1, concerning Therapeutic Protein X: Kertainocyte growth factor 2.
Group 3, all partially as in Group 1, concerning Therapeutic Protein X: C-C Chemokine Receptor 5.
Group 4, all partially as in Group 1, concerning Therapeutic Protein X: Cathepsin K.
Group 5, all partially as in Group 1, concerning Therapeutic Protein X: MPIF-1 (Myeloid Progenitor Inhibitory Factor).
Group 6, all partially as in Group 1, concerning Therapeutic Protein X: VEGF.
Group 7, all partially as in Group 1, concerning Therapeutic Protein X: VEGF-2.
Group 8, all partially as in Group 1, concerning Therapeutic Protein X: BLyS.
Group 9, all partially as in Group 1, concerning Therapeutic Protein X: KD1.
Group 10, all partially as in Group 1, concerning Therapeutic Protein X: TMP-1.
Group 11, all partially as in Group 1, concerning Therapeutic Protein X: TMP-2.
Group 12, all partially as in Group 1, concerning Therapeutic Protein X: TMP-3.
Group 13, all partially as in Group 1, concerning Therapeutic Protein X: TMP-4.
Group 14, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor Protein.
Group 15, all partially as in Group 1, concerning Therapeutic Protein X:CTGF-2.
Group 16, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor 4.
Group 17, all partially as in Group 1, concerning Therapeutic Protein X: Human T-cell Lymphoma-lipoprotein associated phospholipase-A2.
Group 18, all partially as in Group 1, concerning Therapeutic Protein X: VEGI.
Group 19, all partially as in Group 1, concerning Therapeutic Protein X: AIM-1.
Group 20, all partially as in Group 1, concerning Therapeutic Protein X: TNF-delta.
Group 21, all partially as in Group 1, concerning Therapeutic Protein X: TNF-epsilon.
Group 22, all partially as in Group 1, concerning Therapeutic Protein X: AIM-2.
Group 23, all partially as in Group 1, concerning Therapeutic Protein X: Endokine.
Group 24, all partially as in Group 1, concerning Therapeutic Protein X: TR1.
Group 25, all partially as in Group 1, concerning Therapeutic Protein X:TR2.
Group 26, all partially as in Group 1, concerning Therapeutic Protein X: DR3.
Group 27, all partially as in Group 1, concerning Therapeutic Protein X: TR4.
Group 28, all partially as in Group 1, concerning Therapeutic Protein X: 4-1BBsv receptor.
Group 29, all partially as in Group 1, concerning Therapeutic Protein X: OPGL.
Group 30, all partially as in Group 1, concerning Therapeutic Protein X: FasL.
Group 31, all partially as in Group 1, concerning Therapeutic Protein X: Fas.
Group 32, all partially as in Group 1, concerning Therapeutic Protein X: TR5.
Group 33, all partially as in Group 1, concerning Therapeutic Protein X: TR6.
Group 34, all partially as in Group 1, concerning Therapeutic Protein X: DR5.
Group 35, all partially as in Group 1, concerning Therapeutic Protein X: TR8.
Group 36, all partially as in Group 1, concerning Therapeutic Protein X: TR9.
Group 37, all partially as in Group 1, concerning Therapeutic Protein X: TR10.
Group 38, all partially as in Group 1, concerning Therapeutic Protein X: TR11.
Group 39, all partially as in Group 1, concerning Therapeutic Protein X: TR12.
Group 40, all partially as in Group 1, concerning Therapeutic Protein X: TR13.
Group 41, all partially as in Group 1, concerning Therapeutic Protein X: TR14.
Group 42, all partially as in Group 1, concerning Therapeutic Protein X: TR16.
Group 43, all partially as in Group 1, concerning Therapeutic Protein X: HLDOU18.
Group 44, all partially as in Group 1, concerning Therapeutic Protein X: HSDSB09.
Group 45, all partially as in Group 1, concerning Therapeutic Protein X: HDPBQ71.
Group 46, all partially as in Group 1, concerning Therapeutic Protein X: HAGDG59.
Group 47, all partially as in Group 1, concerning Therapeutic Protein X: HCHNF25.
Group 48, all partially as in Group 1, concerning Therapeutic Protein X: HKACD58.
Group 49, all partially as in Group 1, concerning Therapeutic Protein X: HWACB86.
Group 50, all partially as in Group 1, concerning Therapeutic Protein X: HFTCF50.
Group 51, all partially as in Group 1, concerning Therapeutic Protein X: HRDFD27.
Group 52, all partially as in Group 1, concerning Therapeutic Protein X: HCEGG08.
Group 53, all partially as in Group 1, concerning Therapeutic Protein X: HKACI79.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11991

Group 54, all partially as in Group 1, concerning Therapeutic Protein X: HWHGZ51.
Group 55, all partially as in Group 1, concerning Therapeutic Protein X: HDTAI21.
Group 56, all partially as in Group 1, concerning Therapeutic Protein X: HCNCA73.
Group 57, all partially as in Group 1, concerning Therapeutic Protein X: HNHFE71.
Group 58, all partially as in Group 1, concerning Therapeutic Protein X: HLWCF05.

Group 59-117, claim(s) 33, drawn to a method of extending shelf life of Therapeutic Protein X, wherein Therapeutic Protein X can be any of the proteins listed in the Table on page 11 of the description. If any of Groups 59-117 are elected the claim will only be examined in-so-far-as it pertains to the elected Protein X.

The inventions listed as Groups 1-118 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: because the technical feature linking groups 1-118 is not special because Inventions 1-58 do not avoid the prior art as Delta Biotechnology Limited, (EP 322094, June 28, 1989) teaches the claimed sequence. Thus, the invention does not relate to a single inventive concept and is not a contribution over the prior art.

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(71) Applicant *(for all designated States except US)*: **HUMAN GENOME SCIENCES, INC.** [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROSEN, Craig, A.** [US/US]; 22400 Rolling Hill Lane, Laytonsville, MD 20882 (US). **HASELTINE, William, A.** [US/US]; 3053 P. Street, N.W., Washington, DC 20007 (US).

(74) Agents: **GARRETT, Arthur, S.** et al.; Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*

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9 January 2003

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/079480 A1

(54) Title: ALBUMIN FUSION PROTEINS

(57) Abstract: The present invention encompasses albumin fusion proteins. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells. Additionally the present invention encompasses pharmaceutical compositions comprising albumin fusion proteins and methods of treating, preventing, or ameliorating diseases, disorders or conditions using albumin fusion proteins of the invention.

ALBUMIN FUSION PROTEINS

BACKGROUND OF THE INVENTION

The invention relates generally to Therapeutic proteins (including, but not limited to, a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or fragments or variants of albumin. The invention further relates to Therapeutic proteins (including, but not limited to, a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or fragments or variants of albumin, that exhibit extended shelf-life and/or extended or therapeutic activity in solution. These fusion proteins are herein collectively referred to as "albumin fusion proteins of the invention." The invention encompasses therapeutic albumin fusion proteins, compositions, pharmaceutical compositions, formulations and kits. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention using these nucleic acids, vectors, and/or host cells.

The invention is also directed to methods of *in vitro* stabilizing a Therapeutic protein via fusion or conjugation of the Therapeutic protein to albumin or fragments or variants of albumin.

Human serum albumin (HSA, or HA), a protein of 585 amino acids in its mature form (as shown in Figure 15 or in SEQ ID NO:18), is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HA for clinical use is produced by extraction from human blood. The production of recombinant HA (rHA) in microorganisms has been disclosed in EP 330 451 and EP 361 991.

The role of albumin as a carrier molecule and its inert nature are desirable properties for use as a carrier and transporter of polypeptides *in vivo*. The use of albumin as a component of an albumin fusion protein as a carrier for various proteins has been suggested in WO 93/15199, WO 93/15200, and EP 413 622. The use of N-terminal fragments of HA for fusions to polypeptides has also been proposed (EP 399 666). Fusion of albumin to the Therapeutic protein may be achieved by genetic manipulation, such that the DNA coding for HA, or a fragment thereof, is joined to the DNA coding for the Therapeutic protein. A suitable host is then transformed or transfected with the fused nucleotide sequences, so arranged on a suitable plasmid as to express a fusion polypeptide. The expression may be effected *in vitro* from, for example, prokaryotic or eukaryotic cells, or *in vivo e.g.* from a

transgenic organism.

Therapeutic proteins in their native state or when recombinantly produced, such as interferons and growth hormones, are typically labile molecules exhibiting short shelf-lives, particularly when formulated in aqueous solutions. The instability in these molecules when formulated for administration dictates that many of the molecules must be lyophilized and refrigerated at all times during storage, thereby rendering the molecules difficult to transport and/or store. Storage problems are particularly acute when pharmaceutical formulations must be stored and dispensed outside of the hospital environment. Many protein and peptide drugs also require the addition of high concentrations of other protein such as albumin to reduce or prevent loss of protein due to binding to the container. This is a major concern with respect to proteins such as IFN. For this reason, many Therapeutic proteins are formulated in combination with large proportion of albumin carrier molecule (100-1000 fold excess), though this is an undesirable and expensive feature of the formulation.

Few practical solutions to the storage problems of labile protein molecules have been proposed. Accordingly, there is a need for stabilized, long lasting formulations of proteinaceous therapeutic molecules that are easily dispensed, preferably with a simple formulation requiring minimal post-storage manipulation.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that Therapeutic proteins may be stabilized to extend the shelf-life, and/or to retain the Therapeutic protein's activity for extended periods of time in solution, *in vitro* and/or *in vivo*, by genetically or chemically fusing or conjugating the Therapeutic protein to albumin or a fragment (portion) or variant of albumin, that is sufficient to stabilize the protein and/or its activity. In addition it has been determined that the use of albumin-fusion proteins or albumin conjugated proteins may reduce the need to formulate protein solutions with large excesses of carrier proteins (such as albumin, unfused) to prevent loss of Therapeutic proteins due to factors such as binding to the container.

The present invention encompasses albumin fusion proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or a fragment (portion) or variant of albumin. The present invention also encompasses albumin fusion proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or a fragment (portion) or variant of albumin, that is sufficient to prolong the shelf life of the Therapeutic protein, and/or stabilize the Therapeutic protein and/or its activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo*. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors

containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells.

The invention also encompasses pharmaceutical formulations comprising an albumin fusion protein of the invention and a pharmaceutically acceptable diluent or carrier. Such formulations may be in a kit or container. Such kit or container may be packaged with instructions pertaining to the extended shelf life of the Therapeutic protein. Such formulations may be used in methods of treating, preventing, ameliorating, or diagnosing a disease or disease symptom in a patient, preferably a mammal, most preferably a human, comprising the step of administering the pharmaceutical formulation to the patient.

In other embodiments, the present invention encompasses methods of preventing treating, or ameliorating a disease or disorder. In preferred embodiments, the present invention encompasses a method of treating a disease or disorder listed in the "Preferred Indication Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein portion corresponding to a Therapeutic protein (or fragment or variant thereof) disclosed in the "Therapeutic Protein X" column of Table 1 (in the same row as the disease or disorder to be treated is listed in the "Preferred Indication Y" column of Table 1) in an amount effective to treat prevent or ameliorate the disease or disorder.

In another embodiment, the invention includes a method of extending the shelf life of a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) comprising the step of fusing or conjugating the Therapeutic protein to albumin or a fragment (portion) or variant of albumin, that is sufficient to extend the shelf-life of the Therapeutic protein. In a preferred embodiment, the Therapeutic protein used according to this method is fused to the albumin, or the fragment or variant of albumin. In a most preferred embodiment, the Therapeutic protein used according to this method is fused to albumin, or a fragment or variant of albumin, via recombinant DNA technology or genetic engineering.

In another embodiment, the invention includes a method of stabilizing a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) in solution, comprising the step of fusing or conjugating the Therapeutic protein to albumin or a fragment (portion) or variant of albumin, that is sufficient to stabilize the Therapeutic protein. In a preferred embodiment, the Therapeutic protein used according to this method is fused to the albumin, or the fragment or variant of albumin. In a most preferred embodiment, the Therapeutic protein used according to this method is fused to albumin, or a fragment or variant of albumin, via recombinant DNA technology or genetic engineering.

The present invention further includes transgenic organisms modified to contain the

nucleic acid molecules of the invention, preferably modified to express the albumin fusion proteins encoded by the nucleic acid molecules.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the extended shelf-life of an HA fusion protein in terms of the biological activity (Nb2 cell proliferation) of HA-hGH remaining after incubation in cell culture media for up to 5 weeks at 37°C. Under these conditions, hGH has no observed activity by week 2.

Figure 2 depicts the extended shelf-life of an HA fusion protein in terms of the stable biological activity (Nb2 cell proliferation) of HA-hGH remaining after incubation in cell culture media for up to 3 weeks at 4, 37, or 50°C. Data is normalized to the biological activity of hGH at time zero.

Figures 3A and 3B compare the biological activity of HA-hGH with hGH in the Nb2 cell proliferation assay. Figure 3A shows proliferation after 24 hours of incubation with various concentrations of hGH or the albumin fusion protein, and Figure 3B shows proliferation after 48 hours of incubation with various concentrations of hGH or the albumin fusion protein.

Figure 4 shows a map of a plasmid (pPPC0005) that can be used as the base vector into which polynucleotides encoding the Therapeutic proteins (including polypeptide and fragments and variants thereof) may be cloned to form HA-fusions. Plasmid Map key: PRB1p: *PRB1 S. cerevisiae* promoter; FL: Fusion leader sequence; rHA: cDNA encoding HA; ADH1t: *ADH1 S. cerevisiae* terminator; T3: T3 sequencing primer site; T7: T7 sequencing primer site; Amp R: β-lactamase gene; ori: origin of replication. Please note that in the provisional applications to which this application claims priority, the plasmid in Figure 4 was labeled pPPC0006, instead of pPPC0005. In addition the drawing of this plasmid did not show certain pertinent restriction sites in this vector. Thus in the present application, the drawing is labeled pPPC0005 and more restriction sites of the same vector are shown.

Figure 5 compares the recovery of vial-stored HA-IFN solutions of various concentrations with a stock solution after 48 or 72 hours of storage.

Figure 6 compares the activity of an HA-α-IFN fusion protein after administration to monkeys via IV or SC.

Figure 7 describes the bioavailability and stability of an HA-α-IFN fusion protein.

Figure 8 is a map of an expression vector for the production of HA-_-IFN.

Figure 9 shows the location of loops in HA.

Figure 10 is an example of the modification of an HA loop.

Figure 11 is a representation of the HA loops.

Figure 12 shows the HA loop IV.

Figure 13 shows the tertiary structure of HA.

Figure 14 shows an example of a scFv-HA fusion

Figure 15 shows the amino acid sequence of the mature form of human albumin (SEQ ID NO:18) and a polynucleotide encoding it (SEQ ID NO:17).

DETAILED DESCRIPTION

As described above, the present invention is based, in part, on the discovery that a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) may be stabilized to extend the shelf-life and/or retain the Therapeutic protein's activity for extended periods of time in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo*, by genetically fusing or chemically conjugating the Therapeutic protein, polypeptide or peptide to all or a portion of albumin sufficient to stabilize the protein and its activity.

The present invention relates generally to albumin fusion proteins and methods of treating, preventing, or ameliorating diseases or disorders. As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "Therapeutic protein portion" or an "albumin protein portion").

In one embodiment, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein (e.g., as described in Table 1) and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.